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The *Caenorhabditis elegans* death protein Ced-4 contains a motif with similarity to the mammalian 'death effector domain'

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Abstract In the nematode *Caenorhabditis elegans* apoptosis is tightly regulated by a hierarchical set of genes. Two of these, *ced-3* and *ced-9*, possess mammalian homologues encoding executional ICE proteases and inhibitory Bcl-2-related proteins, respectively. The function of a third key player, *ced-4*, is however completely unknown and no mammalian counterparts have been identified. Here we report that Ced-4 protein contains a structural region with similarity to the mammalian death effector domain which has previously been demonstrated to act as an important protein interaction motif in the signaling pathway of the mammalian surface receptor Fas (APO-1, CD95). Based on this finding and previously described genetic experiments, we propose that Ced-4, similar to the mammalian proteins FADD and FLICE, may possess a function as an adaptor protein in invertebrate apoptotic pathways.

Key words: Apoptosis; Ced-4; Fas; APO-1; Death effector domain; FADD; FLICE

1. Introduction

A hallmark of apoptosis is not only that morphological alterations are quite uniform but also that the genetic machinery controlling cell death is highly conserved. In mammalian cells, major inducers of apoptosis include Fas (APO-1, CD95) and TNF-RI, two related surface receptors that mediate cell death in various target cells [1,2]. Both receptors contain an intracellular domain of about 70 amino acids which is essential for transmission of the apoptotic signal and has therefore been called the 'death domain' (DD) [3]. The DD sequence acts as an interface by recruiting other proteins to the receptor. One of these is FADD (MORT-1), a bipartite molecule which in its C-terminus contains a DD sequence mediating DD-DD interaction with the Fas receptor [4,5].

In its N-terminus FADD has a second protein motif which is required to trigger apoptosis and has been designated the 'death effector domain' (DED). The N-terminal part of FADD alone is sufficient for apoptosis, whereas N-terminal truncations of FADD act as dominant-negative inhibitors of Fas-triggered apoptosis [4,5]. Thus, the DED sequence defines a protein interaction motif that through the adaptor protein FADD couples distal effector molecules to the receptor. Very recently, two groups have succeeded in the identification of such a receptor-associated effector component, called FLICE (MACH) [6,7]. FLICE contains two tandem DED regions with homology to FADD that mediate FADD/FLICE interaction. At its C-terminus FLICE possesses a proteolytically active region homologous to the interleukin-1β-converting enzyme (ICE) proteases. ICE proteases are an emerging family of cysteine proteases that have been implicated as the key executioners in apoptosis [8–10]. It is assumed that recruitment of FADD to the receptor somehow leads to processing of the inactive FLICE precursor to the active protease. Activation of FLICE may then trigger one or more other ICE proteases resulting in the final destruction of the cell's architecture.

Important for our understanding of apoptosis was the finding that the basic machinery controlling cell death is highly conserved in mammals and invertebrates. Of the three genes that regulate apoptosis in *Caenorhabditis elegans*, two, *ced-3* and *ced-4*, are required for the execution of cell death [11]. In contrast, another gene, *ced-9*, protects cells that normally survive from undergoing programmed cell death. Loss-of-function mutations in *ced-9* cause cells that normally live instead of undergoing programmed cell death. The excess cell deaths require the activity of *ced-3* and *ced-4*, indicating that *ced-9* acts by preventing *ced-3* and *ced-4* from causing cell death.

Both *ced-9* and *ced-3* have known mammalian counterparts that function in cell death. *Ced-9* is the structural and functional homologue of *bcl-2*, one of a family of genes intimately involved in prevention of vertebrate apoptosis [12]. *Ced-3* encodes a protease related to ICE members, the essential executioners of apoptosis in mammals [13]. In contrast, the function of *ced-4* is completely obscure and as yet no mammalian homologue has been identified [14]. Mutations in either *ced-3* or *ced-4* block all naturally occurring cell death in *C. elegans*, suggesting that both gene products may converge on the same signaling pathway [15].

In this report we have performed a detailed sequence analysis of the Ced-4 protein. It is demonstrated that Ced-4 contains a region with similarity to the DED sequence of the recently identified mammalian proteins FADD and FLICE. We propose that, in analogy the mammalian apoptotic pathway, Ced-4 may act as an adaptor protein activating Ced-3 protease activity during execution of invertebrate cell death.

2. Materials and methods

Protein sequences were retrieved from the GenBank and Swiss-Prot databases. Homology searches were performed using the BLAST and FASTA programs which were accessed by WWW servers. Alignments were constructed using the BLOSUM 45 and other available similarity matrices with default gap penalties. Statistical significance of similarities was evaluated by regional shuffling and the MACAW 2.0.3. algorithm [16]. Secondary structures were predicted using the PHDsec program [17] which is available at the EMBL server.

3. Results and discussion

Recent genetic analysis has shown that overexpression of

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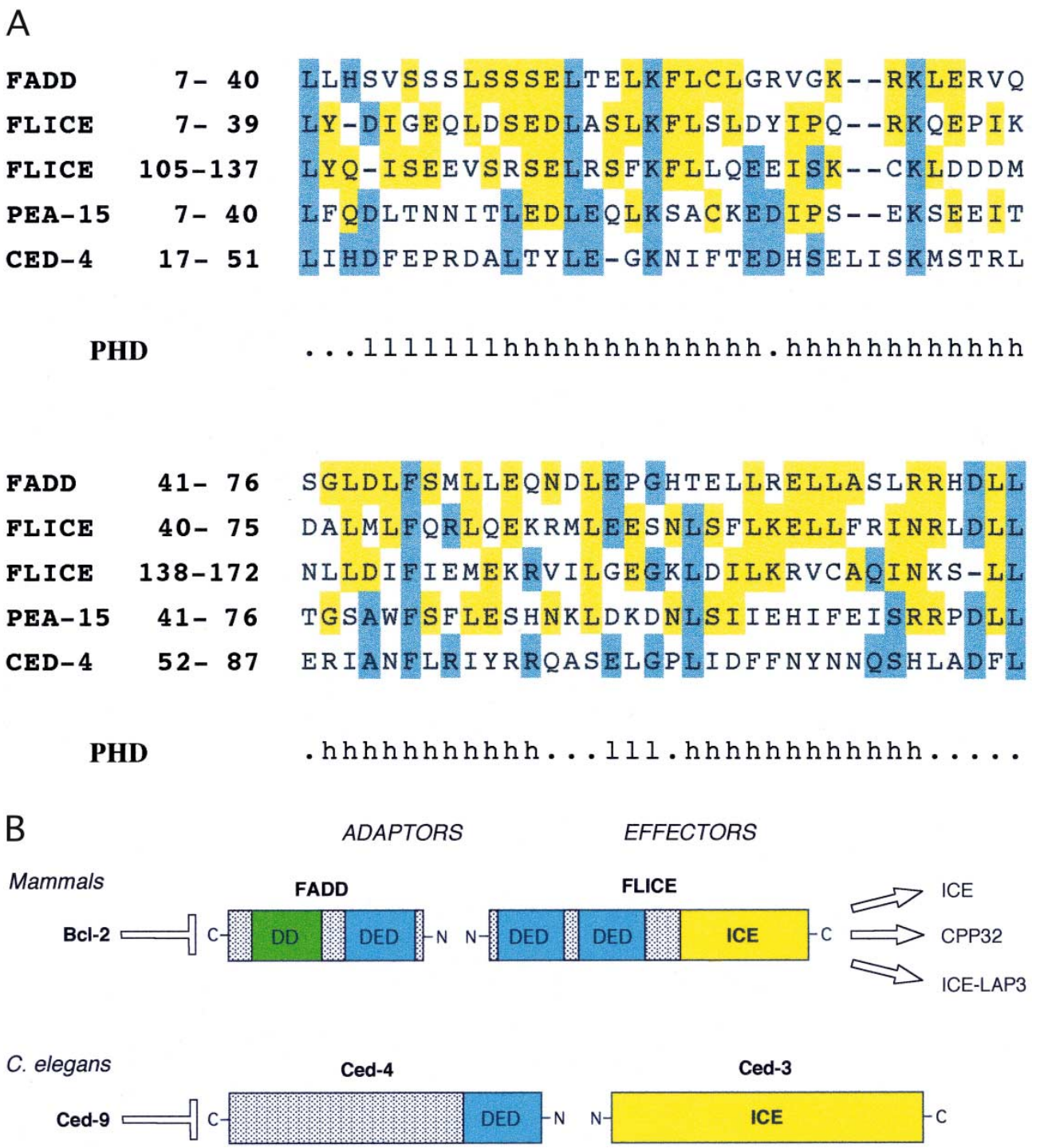

Fig. 1. A: Sequence alignment of Ced-4 (N-terminal region) with the death effector domains (DED) of human FADD (MORT-1), FLICE (MACH) and PEA-15. Numbers give the position of amino acids in each sequence. Amino acids identical in two sequences are marked by blue boxes if including Ced-4, and in yellow boxes if not including Ced-4. The secondary structures were predicted using PHD [16] and are shown beneath the alignment (l, loop; h, α-helix). The Genbank and Swiss-Prot accession numbers were U24231 for human FADD, U58143 for human FLICE, X86694 for PEA-15, and X69016 for Ced-4. B: Proposed functional analogy of apoptosis in mammals and *C. elegans*. In mammalian cells, activation of the surface receptors Fas and TNF-RI triggers an apoptotic signal which results in activation of FLICE and other ICE proteases, such as ICE, CPP32 and ICE-LAP3. The signal is transduced through recruitment of FADD and FLICE to the receptor. The interaction of Fas and FADD is due to the association of their respective death domains (DD). In turn, FADD recruits FLICE through binding to the death effector domains (DED) and somehow activates FLICE proteolytic activity. In *C. elegans*, the functional homologue of ICE proteases is Ced-3. Ced-4 is thought to act upstream of Ced-3, possibly through its DED motif. Apoptosis is prevented by the Bcl-2 homologue Ced-9 which presumably inhibits Ced-4 activity.

ced-4 requires *ced-3* function, whereas overexpression of *ced-3* induces cell death independent of *ced-4* [15]. This indicates that *ced-4* acts presumably upstream of *ced-3*. Prompted by this hierarchical order of the *C. elegans* death genes and the

recent elucidation of the proximal Fas signaling pathway, we performed a detailed analysis of the Ced-4 protein sequence.

When Ced-4 was used for the database searches, no similarity was found to mammalian death proteins. A closer inspection of the N-terminal region of Ced-4, however, revealed a similarity to the DED motif present in the Fas signaling components FADD and FLICE (Fig. 1A). Another DED-containing protein, PEA-15, which has been identified as an astrocyte phosphoprotein of unknown function, contains an even higher degree of similarity. In the N-terminal part of Ced-4, a total number of 14 amino acids out of 71 are identical to PEA-15. Calculation of the probability of matching by chance using MACAW [15] yielded *P*-values in the range of 10^{-2} to 10^{-5}. In addition, when gaps were inserted in the alignment as shown in Fig. 1A, a *P*-value of 6.8×10^{-18} was computed.

It is noteworthy that in all sequences the similarities are localized near the N-terminus. Using the PHD program [16] a regular secondary structure of the aligned sequences was predicted consisting mainly of α-helices in all proteins but not of β-strand structures (Fig. 1A).

The presence of a similar DED motif in Ced-4 therefore raises the possibility that Ced-4 acts as an adaptor and activator protein of Ced-3. Activation of Ced-4 could mediate protein interaction analogous to FADD and the N-terminus of FLICE and finally trigger Ced-3 protease activity (Fig. 1B). This hypothesis is supported by genetic analysis demonstrating that Ced-4 activity depends on Ced-3 as a distal component in the apoptotic pathway [15]. Furthermore, a mutation in the putative DED region of Ced-4 has been reported to abrogate apoptosis [14].

Although the functional importance of the DED-related sequence in Ced-4 has not yet been proven, further structural analysis should clarify the role of Ced-4 as an upstream activator of Ced-3 and functional homologue of FADD. In summary, our finding further supports the notion that the effector mechanisms of apoptosis are highly conserved in invertebrate and mammalian cells.

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