

The putatively protective *Onchocerca volvulus* neuronal protein E1 is a member of the death domain protein family

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Abstract Here we show that E1, an ankyrin-related, potentially protective, neuronal protein of the human filarial nematode *Onchocerca volvulus* contains a death domain (DD), most similar to that of human Mort1/FADD (39% identity). In addition, sequence comparison of E1 to its homologue from *Litomosoides sigmodontis* and to *Caenorhabditis elegans* ankyrin defines two further putative functional domains. One represents the end of the spectrin-binding domain of ankyrins, the other an unique domain, most highly conserved between these nematodes, containing a calpain sequence motif. Thus, E1 may be involved in apoptosis, raising the possibility that protection against this parasitic helminth may be induced by apoptotic processes.

Key words: Helminth; Parasite; Ankyrin; Apoptosis; Neuronal protein; *Onchocerca volvulus*

1. Introduction

Apoptosis induced by Fas/Apo1 and TNF-R is dependent on a so-called death domain (DD), a sequence motif of approx. 65 amino acids present in the cytoplasmic portion of these molecules, which is necessary and sufficient for the transduction of the cell death signal [1,2]. DDs have recently been identified in several molecules, predominantly in those directly associated with cell death, but also in a few others where involvement in cell death has not yet directly been addressed [3–5]. The latter include human ankyrins, their best known function being attachment of membrane proteins to the cytoskeleton [6], as well as a transcript of the ankyrin gene of the free-living nematode *Caenorhabditis elegans*, which is essential in axon guidance [7].

E1 is an ankyrin-related protein of the filarial nematode *Onchocerca volvulus* [8], the causative agent of human onchocerciasis. E1 has been identified by immunoscreening of an *O. volvulus* cDNA library with sera from persons putatively immune to onchocerciasis. It has homology to human brain ankyrin [9] as well as to an *C. elegans* ankyrin [7]. In *O. volvulus* E1 was primarily localized in the nervous system of the worm [8], including the sensory axons in the cephalic space of this nematode [10], and represents the first cloned neuronal protein of a parasitic nematode. To enable testing of the protective potential of E1 in animal models we have cloned an E1 homologue from the rodent filarial parasite *Litomosoides sigmodontis*. Comparison of this sequence to

that of *O. volvulus* as well as the analysis of the genomic organization of E1 in *O. volvulus* led to the recognition of three putative functional domains, including a DD, raising the possibility that E1 is involved in apoptosis.

2. Material and methods

2.1. Parasites

Adult *O. volvulus* worms were obtained from surgically removed nodules and processed as previously described [8]. *L. sigmodontis* adult worms were kindly provided by Dr. A. Hörauf at the Bernhard Nocht Institute in Hamburg, Germany.

2.2. RT-PCR, PCR and DNA sequencing

After isolation of total *L. sigmodontis* RNA, using an RNA extraction kit (TRIzol Reagent, Gibco, BRL), cDNA was obtained by reverse transcription using oligo-dT primers. PCR of the cDNA, carried out using primers derived from nucleotide positions 468–489 (forward) and 1836–1856 (reverse) of the *O. volvulus* E1 cDNA sequence [8], was followed by cloning of the PCR products into the pCR II vector (TA Cloning kit, Invitrogen, San Diego, CA, USA). To determine the genomic structure of the *O. volvulus* E1 cDNA, genomic DNA was isolated from *O. volvulus* as previously described [8] and PCR amplification was carried out using a primer pair spanning the entire nucleotide sequence of the E1 cDNA. The nucleotide sequences were determined in both orientations using [α -³⁵S]dATP and a sequenase kit (US Biochemical Corp., Cleveland, OH, USA). Searches of the SwissProt protein database were performed using the FASTA program.

3. Results

3.1. cDNA cloning and sequence comparisons

Cloning of the *L. sigmodontis* cDNA corresponding to the *O. volvulus* E1 cDNA and comparison between the respective predicted amino acid sequences indicates that the *L. sigmodontis* sequence is overall highly similar (72% identity) (Fig. 1). However, the similarities are clustered in three major regions, whereby flanking regions are less similar (Fig. 1). Alignment of both sequences with the *C. elegans* ankyrin A049, product of the unc-44 gene [7], confirms that three regions are highly conserved between these nematodes and delineates these domains as shown in Fig. 1. Thus, domain I encompasses 101 amino acids, domain II 61 amino acids and domain III 57 amino acids. Amino acid identities between *O. volvulus* and *L. sigmodontis* are 85% for domain I, 90% for domain II and 93% for domain III, while the intervening sequences are less conserved (50% and 64% identical, respectively). The comparison between the *O. volvulus* and *C. elegans* amino acid sequences indicates identities of 75% for domain I, 54% for domain II and 86% for domain III, the intervening sequences being completely dissimilar (Fig. 1).

Amino acid sequence comparison of the domains with known protein sequences indicates that domain I is highly similar to the C-terminal region of the spectrin-binding do-

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Abbreviations: DD, death domain; p55 TNF-R, the p55 tumor necrosis factor receptor; NGF-R, nerve growth factor receptor; unc-44 gene, ankyrin-related gene of *Caenorhabditis elegans*

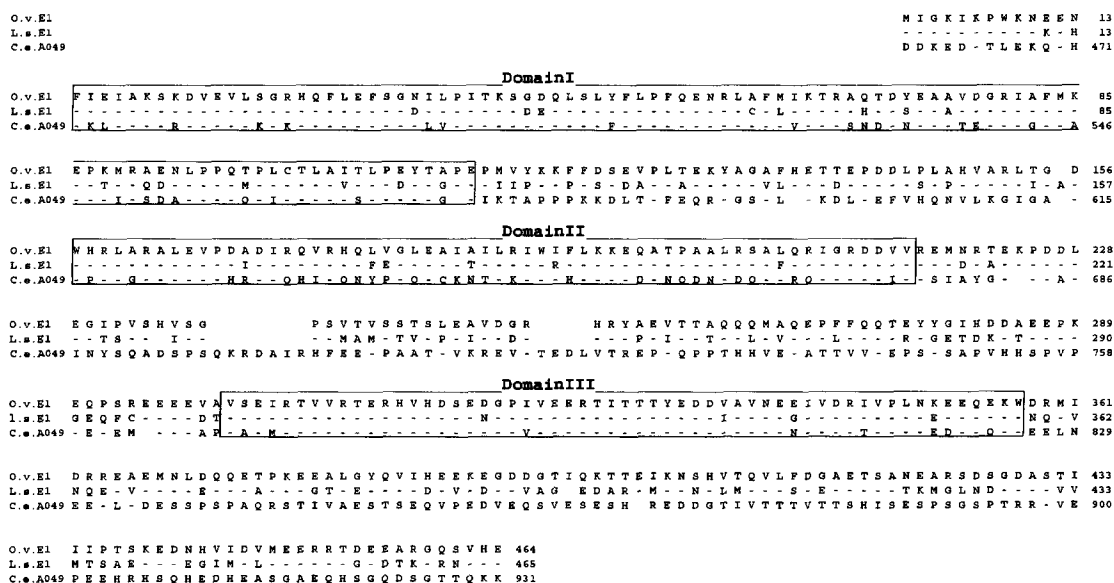


Fig. 1. Alignment of the amino acid sequences of *O. volvulus* E1, its homologue from *L. sigmodontis* and the *C. elegans* A049 ankyrin [7]. The three conserved domains are boxed. Identical amino acids in the *L. sigmodontis* and *C. elegans* sequences are represented by dashes, and gaps, introduced to maximize alignment, are indicated by a blank space.

main of human ankryns [9,11–13] (43% identity to human brain ankryn). Domain II contains a conserved motif known as the death domain (DD) [1,2]. Alignment of the E1 protein sequence with the DD of several proteins is shown in Fig. 2A. Identities between E1 and these human proteins are 30% for ankryn 1 [11], 22% for Fas/APO1 [14], 39% for Mort1/FADD [15,16], 24% for p55 TNF-R [17,18] and 29% for NGF-R [19]. The identity to Mort1/FADD [15,16] is also the highest among two members of the DD protein family [3], whereby the *O. volvulus* ankryn sequence is more identical to the DD of the human Mort1/FADD than to the DD of human ankryns. The valine residue at position 239 of Fas/Apo1 was shown to represent a functional residue [1], since point mutations at this position abolish the ability of cell death signal transduction. This valine residue is conserved in the nematode, but not in the human ankryns (Fig. 1) [9,11–13].

Domain III shows the highest degree of conservation between the three nematode species (Fig. 1). Similarity searches of domain III with protein sequences in the data bank revealed no significant identities over the entire sequence to other proteins. However, a short sequence motif from amino acid 39–56 of this domain which is 65% identical to human and rat calpain (large subunit) [20,21] is found (Fig. 2B).

3.2. Genomic sequence analysis

Analysis of the genomic DNA fragment encoding the *O. volvulus* E1 cDNA indicates that the cDNA is encoded on 9 exons divided by 8 introns (Fig. 2C). In contrast, the corresponding *C. elegans* genomic region contains 3 exons interrupted by 2 introns [7] (Fig. 2C). In *O. volvulus* each of the three domains is encoded on 2 exons (Fig. 2C), whereby in *C. elegans* domain I and II are both encoded on the same large exon [7] (Fig. 2C). In *O. volvulus* domain III is encoded on exons 6 and 7, whereby the boundary of exon 6 precisely matches the boundary of the start of domain III in the protein. The coding sequence for domain III continues on exon 7 which encodes only the following 18 amino acids. In *C. elegans* exon B corresponds exactly to domain III [7] (Fig. 2C).

4. Discussion

The analysis presented here defines three domains which are highly conserved in nematode ankyrins. The first domain is also highly conserved in ankyrins from vertebrates [9,11–13], where it is found at the C-terminal end of the so-called spectrin-binding domain [22]. However, this conserved region is not directly involved in binding to spectrin, and its exact function remains to be determined [23].

Similarly, the third domain recognized here has not yet been functionally characterized. The high degree of conservation as well as its genomic organization in both *O. volvulus* and *C. elegans* strongly suggests that it is functionally important in nematodes. In spite of its almost complete conservation in nematodes we could not detect a corresponding domain in the vertebrate ankyrins known to date. The fact that in nematodes it is precisely encoded on separate exons and is not part of the exons encoding other portions of the ankyrin molecule, such as the spectrin-binding domain, could indicate that in vertebrates this domain is encoded on another, as yet unidentified, gene. It has been suggested that the nematode ankyrin gene is ancestral to the multiple ankyrin genes of vertebrates [7]. Interestingly, splicing events occur in the particular region of mammalian ankyrins where domain III is localized in the nematode ankyrins [9,11–13]. These events result in the presence or absence of different inserts which are not related to the nematode domain III, but which appear related to the function and the tissue specificity of the respective ankyrin [11–13]. If indeed, domain III proves to be nematode-specific, together with its suggested functional importance it could represent a particularly attractive target for immune-mediated protection studies.

A sequence comparison of domain III to proteins in the data bank revealed no significant identities over the entire domain, however one region of this domain is similar to a motif found in the protease calpain, located in the proteolytic site of the enzyme [20,21]. Calpain is a protease with defined substrate specificities which include spectrin, fodrin [24] and

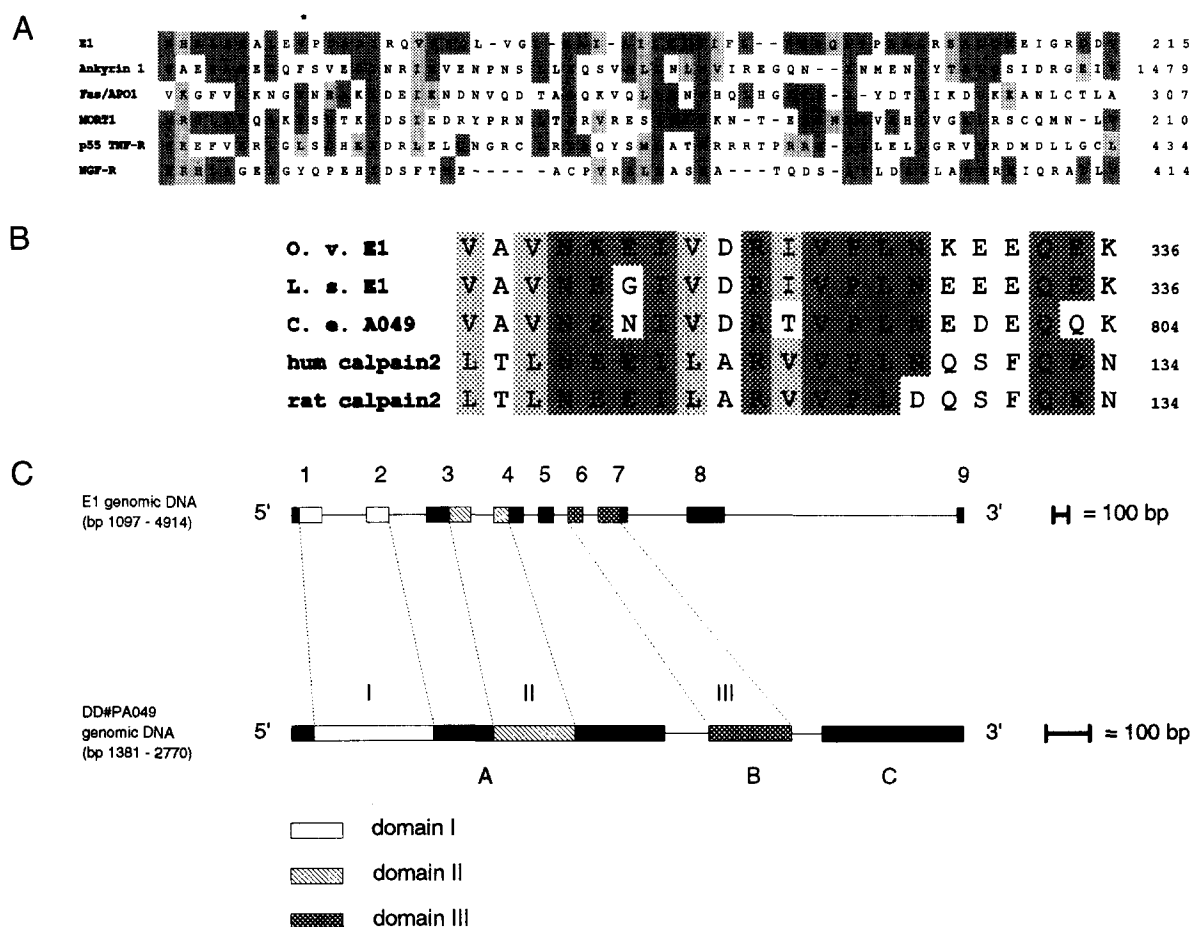


Fig. 2. (A) Alignment of the DDs of *O. volvulus* E1 [8], human ankyrin1 [11], Fas/Apo1 [14], Mort1/FADD [15,16], p55 TNF-R [17,18] and NGF-R [19]. Identical amino acids are boxed and darkly shaded, amino acids considered conserved are boxed and lightly shaded; gaps introduced to maximize alignment are represented by dashes. The position of the valine residue, essential for apoptotic signalling, is indicated by an asterisk. (B) Alignment of the regions of domain III of *O. volvulus*, *L. sigmodontis* and *C. elegans* showing similarity to human and rat calpain [20,21] using the program FASTA. Matches between identical amino acids are shaded in dark, conservative amino acid matches are lightly shaded. (C) Schematic representation of the *O. volvulus* genomic region encoding E1 and comparison with the corresponding genomic fragment of the *C. elegans* unc-44 ankyrin gene [7]. Exons are boxed and numbered 1–9 for the *O. volvulus* sequence and labeled A–C for the *C. elegans* sequence. The locations of domain I–III and of the intervening sequences on the exons are indicated by white or shaded and black boxes, respectively.

ankyrin [25], its activity being associated with cell death [26], particularly during neurodegeneration [27]. Future studies can address whether this domain has proteolytic activity or interacts with other proteins. As this domain is highly conserved in *C. elegans*, mutagenesis studies could be used to elucidate its function.

In contrast to domains I and III, the second domain found in the *O. volvulus* and *L. sigmodontis* ankyrins, corresponds to a functionally characterized protein domain, the so-called DD. The DD is essential for the transduction of the cell death signal in apoptotic molecules [1,2] and is also conserved in human and *C. elegans* ankyrins [3–5]. As shown here, the *O. volvulus* E1 protein as well as its *L. sigmodontis* homologue are members of this DD protein family. A characteristic feature of DDs from different proteins is their ability to self-associate [1,2]. The DD in Mort1/FADD, to which the DD in E1 is most similar, has been shown to associate also with the DDs of other signalling receptors, particularly with the DD of Fas/APO1, suggesting that it may be a mediator of functional cross-talk between different apoptosis receptors early in their signaling cascades [28]. Further studies will ad-

dress whether the DD in E1 is functional in apoptosis and whether it interacts with DDs of other proteins.

E1 as an immunogen for putatively immune individuals together with its localization in the nervous system of the worm [8], including the neuronal structures which are exposed to the environment via the amphids [10], have implicated that antibodies against neuronal structures may be involved in protection against parasitic helminths [8]. The present results address the molecular mechanisms involved in this protective process, raising the question of whether apoptotic mechanisms during neuronal development are involved, and provide an experimental basis for future studies in this direction. Indeed, apoptosis plays a central role in the development of the eukaryotic nervous system [29] and the function of the nervous system appears essential for the development and survival of parasitic nematodes in their hosts [30]. In this regard interference with the neuronal apoptotic pathway may represent an effective strategy to control parasitic helminths, which has not yet been pursued. Induction of apoptosis has been discussed in therapeutic strategies for cancer, autoimmune diseases and viral infections. The present results suggest that this

may also be evaluated in control strategies against parasitic helminths.

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